Persistence of *Schistosoma japonicum* DNA in a Kidney–Liver Transplant Recipient

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Abstract. Mitochondrial genome analysis of *Schistosoma japonicum* suggests that diversity of intermediate host snails drove intra-species divergence during its expansion in Asia. We applied the knowledge of this genomic variation to study an unusual patient we recently diagnosed with schistosomiasis. The patient had not visited any schistosomiasis-endemic countries for more than 35 years and had no idea where she became infected. Unusual clinical features of this patient included the absence of egg granulomas in tissue and persistent noncalcified eggs despite multiple praziquantel (PZQ) treatments over 7 years. A digital droplet polymerase chain reaction (PCR) assay that specifically targets the schistosome 1,4 dihydronicotinamide adenine dinucleotide-1 (NADH1) dehydrogenase-1 mitochondrial gene successfully amplified parasite DNA extracted from colon biopsies. DNA sequence analysis of parasite DNA revealed that it was a Philippine strain of *S. japonicum*. Future molecular studies using stored DNA from patients such as this may provide new insight into why some persons do not respond well to PZQ treatment.

Schistosomiasis is an important neglected tropical parasitic disease that affects more than 250 million persons worldwide.^{1,2} On rare occasions, it has been reported that humans can remain asymptomatic for decades before diagnosis and treatment.³ Emerging issues in schistosomiasis control include concern for development of praziquantel (PZQ) resistance or reduced efficacy, and evolution of new hybrid schistosomes that can infect humans.^{4,5} Mitochondrial genome analysis of *Schistosoma japonicum* in Asia suggests that diversity of intermediate host snail vectors drove intra-species divergence of the parasite during its regional expansion.^{6,7} We applied knowledge of schistosome genomic variation to study a unique patient we recently diagnosed with schistosomiasis and progressive end-stage liver disease in 2010. The patient had not visited any endemic countries since 1975 but as a child had lived in several Asian and Southeast Asian countries including the Philippines, China, and South Korea. She had no known exposure to schistosomes in the United States and had no idea where she became infected.

At presentation in 2010, the patient had multiple stool samples for ova and parasites that were negative, and serology for schistosomiasis performed twice by a regional reference laboratory were negative. Esophagogastroduodenoscopy showed esophageal varices, and colonoscopy revealed that the entire length of the colon was tubular, pale, and friable. Computerized axial tomography imaging revealed massive splenomegaly and extensive periportal venous collaterals consistent with portal hypertension. Liver biopsy revealed multifocal bridging fibrosis and numerous round to oval noncalcified ova with no egg granulomas. Noncalcified ova without granulomas also were present in the colonic mucosa and submucosa. Tissue biopsies were reviewed by the CDC, and the morphology of eggs was irregular but consistent with a species of schistosome.

Despite multiple high-dose PZQ treatments between 2010 and 2017, liver and kidney disease progressed and repeat colon biopsies demonstrated noncalcified eggs with no granulomas. This scenario raised several questions: First, was she infected with a human or nonhuman species of schistosome that was

relatively PZQ resistant? Were the eggs already dead but noncalcified and morphologically intact? Could she have been infected with a human–animal schistosome hybrid with reduced susceptibility to PZQ? Was there an unidentified immunological defect that prevented egg granuloma formation? Because egg granulomas are expected in schistosomiasis,

FIGURE 1. (A) Gross appearance of explanted liver from patient with *Schistosoma japonicum* infection. (B) Histological findings in liver and subsequent colon biopsies demonstrated noncalcified ova without granuloma formation (magnification $\times 400$). This figure appears in color at www.ajtmh.org.

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FIGURE 2. (A) Phylogenetic tree constructed using the maximum likelihood method to compare *Schistosoma japonicum* strains based on mitochondrial NADH1 sequences. Included in this comparison are sequences from the patient, *S. japonicum* from China (Hunan, Jiangxi) and the Philippines (Sorsogon, Mindoro, Leyte), *Schistosoma mekongi*, *Fasciola gigantica*, and *Schistosoma bovis*. The phylogenetic tree demonstrates that the patient's parasite is most closely related to *S. japonicum*. This method does not take into account the single-nucleotide polymorphisms used to design PCR primer pairs (SJ1 and SJ2) that distinguish between the Chinese and Philippine strains of *S. japonicum*. Accession numbers are listed for each strain at the left of the sequence. (B) DNA sequence alignments representing *S. japonicum* strains from China (Jiangxi, Hunan) and the Philippines (Leyte, Sorsogon, Mindoro) compared with the DNA sequence obtained from the patient. Single-nucleotide polymorphisms (highlighted in gray) in specific regions of the NADH1 dehydrogenase one mitochondrial gene allow for the design of PCR primer sets that differentiate Chinese *S. japonicum* from *S. japonicum* found in the Philippines. Accession numbers are listed at the left of each sequence. (C) Agarose gel electrophoresis of PCR products identified *S. japonicum* DNA in two of three colonoscopy biopsy samples (lanes 3 and 4) using SJ2 primers but no amplicons were generated using SJ1 primers (lanes 5, 6, and 7) that are specific for Chinese *S. japonicum*. Lane 1 contains positive control PCR products using SJ2 primers with Philippine *S. japonicum*, and Lane 8 shows positive control PCR products obtained with SJ1 primers and *S. japonicum* DNA from China. DNA molecular weight markers are displayed on the far left.

several immunological studies were completed in addition to standard pre-transplant testing. An anti-cytokine antibody panel was negative, as was testing for Mendelian susceptibility to Mycobacterial Disease, and the patient exhibited normal activation of interferon gamma stimulated signal transducer and activator of transcription-1 (STAT-1) in monocytes. Flow cytometry confirmed mild lymphopenia.

In 2016, the patient required a combined kidney–liver transplant. Serological testing by the CDC identified the antibody for *S. japonicum* and/or *Schistosoma mekongi* by immunoblot. The explanted liver was formalin fixed and demonstrated extensive fibrosis and noncalcified eggs (Figure 1). After transplantation, the patient continued to experience recurrent colitis and colon biopsies in 2017 again demonstrated noncalcified ova. At this point, informed consent was obtained and human subject ethics research approval was obtained to extract parasite DNA from new ethanol-fixed colon biopsies (Medical College of Wisconsin project number PRO00028065). Biopsies were shipped to the Molecular Parasitology Laboratory QIMR Berghofer Medical Research Institute, Brisbane, Australia, for DNA analysis.

A Qiagen DNA Micro kit was used for extraction of genomic DNA from laser-microdissected colon biopsy tissue. The DNA was used in a digital droplet PCR (ddPCR) assay with primers which amplify the NADH1 dehydrogenase I (NADH1) mitochondrial gene of *S. japonicum* as described previously.^{8–13} After amplification, reactions were read using a QX200 (Bio-Rad, Hercules, CA) droplet reader. Non-template controls using water in place of DNA, and positive controls using DNA from *S. japonicum* eggs isolated from the liver of an infected mouse were used in all PCR assays. Conventional PCR was performed on positive ddPCR-positive samples using primers SJ1 and SJ2 which amplify a segment of the NADH1 gene.¹⁰ Conserved single nucleotide polymorphisms in this region of Chinese *S. japonicum* allow design of PCR primer sets (SJ1, SJ2) that differentiate the Chinese and Philippine strains of *S. japonicum* as described previously.⁸ Amplified products were sequenced at Berghofer using the in house Big Dye terminator sequencing service. Sequences were viewed in Finch TV 1.4.0 (Geospiza) and analyzed in Sequencher 5.4.6 (Gene Codes). The DNA sequence of the patient's parasite was analyzed using BLASTn (nucleotide-nucleotide Basic Local Alignment Tool [BLAST], National Center for Biotechnology Information) and compared with known sequences of *S. japonicum* from various locations in China and the Philippines, *S. mekongi*, *Schistosoma bovis*, and *Fasciola gigantica*. A phylogenetic tree of the DNA sequences was constructed using the maximum likelihood method (Figure 2A) that it was concluded that the unknown parasite was *S. japonicum* and not nonhuman *S. bovis* or *S. mekongi*. Alignment of NADH1 segments from the Philippine and Chinese strains of *S. japonicum* demonstrate conserved single nucleotide polymorphisms from which the SJ1 and SJ2 primers are designed (Figure 2B). No amplification of DNA occurred with the SJ1 primers (Chinese *S. japonicum*), but two of three samples produced amplicons of the expected size (242 bp) using the SJ2 primers (Philippines *S. japonicum*, Figure 2C).

In 1975, more than 24 provinces throughout the Philippines reported endemic *S. japonicum*. *Schistosoma japonicum* remains endemic in the Philippines for a variety of reasons, including logistic issues with drug delivery to rural areas/islands, reinfection, and the existence of animal reservoirs of *S. japonicum*, predominantly cattle and carabao.¹⁴ Two negative serological tests for schistosomiasis in this patient

emphasize the difficulties inherent with serodiagnosis of *S. japonicum*. At the CDC, a Falcon Assay Screening Test–Enzyme Linked Immunosorbent Assay (FAST-ELISA) method to detect schistosome antibody is 99% specific for *Schistosoma mansoni* and 90% specific for *Schistosoma hematobium*, but detection of specific antibody against *S. japonicum* and/or *S. mekongi* requires the use of an immunoblot and different adult antigen preparations.¹⁵

The absence of calcified eggs or egg granulomas in this patient was unusual. The noncalcified eggs were most likely dead, and viability could have been tested had the explanted liver not been formalin fixed. Although most adult schistosomes normally live 8–10 years, rare occurrences have been reported where adult worms shed eggs for several decades.³ In immunocompetent animals and humans, calcification of eggs can take 2 years or more, but there is limited information in immunocompromised hosts. It was unlikely, but conceivable, that some eggs were still viable, hence providing a rationale for the multiple PZQ treatments. Concern about putative PZQ resistance in humans gains some support from reports of low cure rates in some human populations.¹⁶ Praziquantel resistance has been induced by repeated subtherapeutic drug exposure in a murine model of *S. japonicum*.¹⁷ Praziquantel-resistant strains of *S. japonicum* produce more eggs than drug-susceptible isolates. To date, there are no accepted genetic markers of reduced PZQ efficacy in animals or humans.

In conclusion, we successfully applied knowledge of schistosome mitochondrial genome variability to identify the country in which a patient became infected decades previously. Analysis of NADH1 sequences also determined the unknown species to be *S. japonicum* that originated in the Philippines, and was not *S. mekongi* nor related to the nonhuman schistosome species *S. bovis*. The patient is doing well 2 years post liver–kidney transplantation with no clinical indications for repeat biopsies of colon or transplanted liver. Immunological studies could find no explanation for the lack of egg granuloma formation in the liver or colon, and we anticipate that all adult schistosomes and eggs are now dead. In the future, if there are genetic markers for decreased efficacy of PZQ, further analysis of stored DNA from this patient may help to address unanswered questions.

Received September 14, 2018. Accepted for publication November 26, 2018.

Published online January 7, 2019.

Acknowledgments: We would like to thank the transplant hepatology and nephrology teams at Froedtert Hospital and the Medical College of Wisconsin for their cooperation. Ayesha Farooq and Kiyoko Oshima, Department of Pathology at Froedtert Hospital, provided photographs of the explanted liver and photomicrographs of representative histological sections. This work was supported in part by Froedtert Hospital and the Medical College of Wisconsin, the QIMR Berghofer Medical Research Institute and the National Health and Medical Research Council of Australia (DPM).

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